International Journal of Fisheries and Aquatic Sciences 2(1): 13-17, 2013
ISSN: 2049-8411; e-ISSN: 2049-842X

Submitted: October 08, 2012 Accepted: December 03, 2012 Published: February 20, 2013

Toxicity of Alcoholic Leaf Extracts of *Lantana indica* Plant: Effect on Haematological and Physiological Parameters in Non-target Fish *Heteropneustes fossilis*

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Abstract: The alcoholic leaf extract of *Lantana indica* Roxb. For the one, two or three weeks induced a number of haematological alterations in the freshwater, Indian cat fishes *Heteropneusts fossilis* (Bloch). It has been shown that leucocytes differential counts were decreased significant compared to controls, serum total proteins, serum glucose, serum total lipid, serum total cholesterol and serum creatinine. *Heteropneustes fossilis* is a common fish of Indian captured fishery its distributed to freshwater of India, Pakistan, Ceylon, Burma and China. The results showing after 24 h exposure of alcoholic leaf extracts of *Lantana indica* against *Heteropneustes fossilis* treated with doses of 7.0 mg/L caused 23% mortality and 9.0, 11.0 and 13.0 mg/L caused 53, 75 and 96%, mortality and after exposure to doses up to 48, 72 and 96 h, in laboratory conditions there was no mortality, respectively. Results concerning red blood cell counts, exhibited a decrease in their numbers after three weeks from the control group (2575.6±4.13) to (154.0±0.180) in alcoholic leaf extracts treated freshwater fish *Heteropneustes fossilis*.

Keywords: Haematology, *Heteropneustes fossilis*, *Lantana indica*, toxicity

INTRODUCTION

With growing awareness of environmental pollution by pesticides, efforts are being made to find pesticides from plant origin (Mian and Mulla, 1992; Arasta*et al*., 1996; Singh *et al*., 1996) because natural compounds are ecologically sound and culturally more acceptable than synthetic pesticides. Earlier reports also indicated that a number of plant families have furnished many classes of natural products, as saponins, tannins, alkaloids, alkenyl phenols, di-and triterpenoids etc., have high pesticidal activity and used to control diseases causing insects such as mosquito larvae, freshwater snails and predatory fish (Marston and Hostettmann, 1985; Singh *et al*., 1996; Srivastava*et al*., 2007).

Despite all the advantages, we are interested in knowing the mode of action and long-term effect of plant origin pesticides on non-target animals, because it cannot be put to commercial use without a study of these aspects as well.

The plant *Lantana indica* (Family: *Verbinaceae*) commonly known as “Indian Lantana” or “Wild Sage”. The leaves of *Lantana indica* are regarded as a cure for snakebite. Its different parts are used in traditional medicine for the treatment of the various human ailments such as ulcer, eczema eruption, malaria and rheumatism (Kirtikar and Basu, 1961; Sastri, 1962; Begum *et al*., 1995; Srivastava *et al*., 2007).

The aim of this study is to present toxicological information about common plant *Lantana indica* Roxb. Against freshwater fish *Heteropneustes fossilis*. Srivastava *et al*. (2007) reported that the *Lantana indica* is a common medicinal plant of India having high molluscicidal and mosquito larvicidal activity in laboratory. *Heteropneustes fossilis* is an important fish of Indian captured fishery and a good experimental material due to its size, easy availability and survivability in laboratory conditions.

MATERIALS AND METHODS

Collection and storage of experimental materials: The freshwater fish *Heteropneustes fossilis* is commonly known as singhi was collected locally from Ramgarh lake of Gorakhpur District, India (Year 2008-09). The netted fish were brought in plastic bucket and released in to tank containing de-chlorinated tap water. Prier to experiment fish was allowed to acclimate to laboratory conditions for seven days. Diseased, injured and dead fish (if any) were removed to prevent the decomposition of the body in the tank. Water was change every day. Fish were fed with commercial food. Acclimatized fish were used for experiments.

Collection and preparation of alcoholic extract of leaves: The leaves of *Lantana indica* were collected locally from Botanical garden of D.D.U. Gorakhpur

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University, Gorakhpur, India. Two kilograms leaves were washed with water dried in incubator at 37°C and powdered with the help of a mechanical device. The dried powder (50 g) was extracted through Soxhlet apparatus in 200 mL of alcohol for about 20 h and after extraction the solvent was evaporated using vacuum pump to obtain the extract in dried form. Dried residue of alcohol extract was stored in airtight desiccators and used for experiments.

Toxicity experiments: In laboratory conditions, toxicity experiments were performed by the method of Singh and Agarwal (1988). Ten experimental animals were kept in glass aquaria containing 10 L of de-chlorinated tap water. Fishes were exposed for 24 to 96 h.

In pond conditions the experiments were performed in freshwater cemented earthen ponds of 29.28 m^2 in area and 9.19 m^3 in water volume. Each cemented earthen pond treated with different concentrations of plant extracts was stocked with fishes. Water analysis for various physico-chemical parameters, viz. temperature, pH, dissolved O_2, free CO_2 and total alkalinity. Water temperature ranged from 27.4-28.6°C. The other parameters were within the following range: total alkalinity 43-62 ppm, pH 6.8-7.7, dissolved oxygen 7.8-10.3 mg/L (APHA, 1992).

Sampling procedure: At weekly intervals blood were withdrawn from the caudal peduncle without any type of anaesthesia. For haematological determination (erthroctic count, haemoglobin content, haematocrit value and leucocytic count) was treated with heparin as anticoagulant and held to be immediately analyses. Serum obtained by centrifugation of the blood at 8000 rpm for 3-5 min, packed cells were discarded and serum was stored in the freezer at -20°C for the determination of total protein, glucose, total lipid, total cholesterol and creatinine.

Hematological procedure: For RBC count Neubaur haemocytometer and Dacie's fluid (Dacie and Lewis, 1968) were used. Haemoglobin content was measured by the alkali haemtin method (Oser, 1965). Haematocrits were estimated by the microhaematocrit method. Total WBC count was estimated with shawn's solution Shaws solution (Shaw, 1930). Differential WBC counts were made on blood smears stained with May-Giemsa's methods.

Methods for determination of non cellular constituents: Total serum protein was determined according to Lowry method (Lowry *et al*., 1951) and modification described by Tsuyoshi and Jaws (1978). Serum glucose was estimated according to the method of Siest *et al*. (1981). Serum total lipid was determined by the method of Frings *et al*. (1972). For the estimation of serum cholesterol and creatinine the method of Clerch and Miale (1970) was applied respectively.

Statistical analysis: The results obtained in the present study were statistically analyzed according to the method of Arkin and Cotton (1963).

RESULTS

The behavioral changes the administration of *Lantana indica* leaf extracts were assumed as reduced activity and even extreme sluggishness. The fish progressively showed a sign of tiredness. Finally they became totally inactive and lay on the bottom of the aquaria and earthen cemented pond. Mortality was almost negligible during the whole of the experimental period between fish treated with either alcoholic leaf extract of *Lantana indica*.

The results showing in Table 1 after 24 h exposure of alcoholic leaf extracts of *Lantana indica* against *Heteropneustes fossilis* treated with doses of 7.0 mg/L caused 23% mortality and 9.0, 11.0, 13.0 mg/L caused 53, 75 and 96% mortality and after exposure to doses up to 48, 72 and 96 h in laboratory conditions there was no mortality, respectively. In experimental pond after 24 h exposure of alcoholic leaf extracts of *Lantana indica* against *Heteropneustes fossilis* treated with doses of 20.0 mg/L caused 18% mortality and 25.0, 30.0, 35.0 mg/L caused 45, 75 and 91% mortality and after exposure to doses up to 48, 72 and 96 h in experimental pond condition conditions there was no mortality, respectively (Table 1). The Erythrocyte count, haemoglobin content and haematocrit value of freshwater fish *Heteropneustes fossilis* after one, two or three weeks exposure of doses of alcoholic leaf extracts of *Lantana indica* plant the erythrocyte count, haemoglobin content and haematocrit value decrease in their numbers (Table 2) after three weeks from the control group.

Results concerning red blood cell counts, exhibited a decrease in their numbers (Table 3) after three weeks from the control group (2575.6±4.13) to (154.0±0.180) in alcoholic leaf extracts treated freshwater fish *Heteropneustes fossilis*. Haemoglobin content of fish *Heteropneustes fossilis* exposed to leaf extract of *Lantana indica* indicated a significant decline in all treated groups exposed to the leaf extracts of *Lantana indica* plant during all the administration period (Table 3). A total and differential leucocyte counts are shown in Table 3, the leaf extract of *Lantana indica* caused a highly significant decrease in total leucocyte count compared to the control group.

In general lymphocytes increased ($p<0.001$) monocytes and neutrophilis decreased ($p<0.001$), A depression in serum total protein administration of leaf extracts of *Lantana indica* was observed between fish

Table 1: Percent mortality of freshwater fish *Heteropneustes fossilis* at different concentrations of alcoholic leaf extracts of *Lantana indica* plant in laboratory conditions and in experimental pond after 24 to 96 h exposure periods

	Laboratory conditions					Experimental ponds			
Dose (mg/L)	24 h	48 h	72 h	96 h	Dose (mg/L)	24 h	48 h	72 h	96 h
Control	-	-	-	-	Control		-	-	-
7.0	23.3±2.31	-	-	-	20.0	18.3±3.37	-	-	-
9.0	53.3±2.31	-	-	-	25.0	45.0±2.45	-	-	-
11.0	75.0±2.45	-	-	-	30.0	75.0±2.45	-	-	-
13.0	96.6±2.31	-	-	-	35.0	91.6±3.37	-	-	-

Mortality data are mean±S.E. of six replicates; Concentrations given are the final concentration (w/v) in aquarium and pond water; -: No mortality was observed

Table 2: Erythrocyte count, haemoglobin content and haematocrit value of freshwater fish *Hetropneustes fossilis* after one, two or three weeks exposure of sub-lethal doses of alcoholic leaf extracts of *Lantana indica* plant

Exposure period	Experimental group	Erythrocyte count (10^6/mm^3)	Haemoglobin content (g/dL)	Haematocrit value (%)
One week	Control	2.56±0.65	16.8±0.12	42.8±0.16
	3.0 mg/L	2.23±0.62	10.5±0.38	36.6±0.22
	5.0 mg/L	1.89±0.62	7.8±0.31	17.5±0.12
Two weeks	Control	2.57±0.65	15.6±0.67	40.4±0.08
	3.0 mg/L	2.15±0.08	7.3±0.86	30.6±0.09
	5.0 mg/L	1.67±0.58	5.5±0.08	27.8±0.39
Three weeks	Control	2.63±0.81	15.0±0.40	40.8±0.10
	3.0 mg/L	2.06±0.01	6.0±0.40	30.8±0.23
	5.0 mg/L	1.41±0.01	4.3±0.40	26.6±0.14

Data are mean±SE of six replicates

Table 3: Leucocyte and its differential counts of freshwater fish *Hetropneustes fossilis* after one, two or three weeks exposure of different sub-lethal doses of alcoholic leaf extracts of *Lantana indica* plant

			Differential count (per mm^3)		
Exposure period	Experimental group	Leucocyte count (per mm^3)	Lymphocyte	Monocyte	Neutrophil
One week	Control	2575.6±4.13	2021.3±1.98	405.0±2.45	154.0±0.80
	3.0 mg/L	2353.3±4.03	1938.3±4.21	283.3±1.22	136.3±1.00
	5.0 mg/L	2062.3±6.29	1705.0±4.01	250.6±1.22	109.0±0.80
Two weeks	Control	2594.0±6.05	2012.0±3.02	420.3±1.83	162.5±0.93
	3.0 mg/L	2137.6±5.47	1806.3±5.01	222.0±1.50	111.8±0.72
	5.0 mg/L	1577.6±3.01	1331.3±2.31	196.1±0.77	47.0±0.63
Three weeks	Control	2419.0±4.93	1991.3±2.60	422.8±0.91	146.6±4.03
	3.0 mg/L	2087.0±9.08	1872.5±3.09	125.0±0.40	92.3±1.05
	5.0 mg/L	1266.6±6.58	1123.6±2.42	115.0±0.80	27.5±0.47

Data are mean±S.E. of six replicates

Table 4: Levels of non-cellular blood constituents of freshwater fish *Hetropneustes fossilis* after one, two or three weeks exposure of different sub-lethal doses of alcoholic leaf extracts of *Lantana indica* plant

Exposure period	Experimental group	Serum total protein (g/dL)	Serum glucose (mg/dL)	Serum total lipid (g/L)	Serum total cholesterol (mg/dL)	Serum creatinine (mg/dL)
One week	Control	6.30±0.02 (100)	92.0±1.26 (100)	10.75±0.04 (100)	180.0±1.85 (100)	1.12±0.08 (100)
	3.0 mg/L	6.00±0.15 (95)	85.0±2.20 (92)	12.86±0.42 (119)	182.0±6.50 (101)	2.25±0.15 (201)
	5.0 mg/L	5.15±0.15 (82)	72.5±1.25 (79)	14.72±0.38 (137)	190.5±3.50 (106)	2.45±0.18 (219)
Two weeks	Control	6.35±0.04 (100)	91.5±1.35 (100)	10.58±0.06 (100)	182.5±1.75 (100)	1.18±0.06 (100)
	3.0 mg/L	5.92±0.06 (93)	80.5±1.25 (88)	13.96±0.56 (132)	198.5±5.40 (109)	2.86±0.18 (242)
	5.0 mg/L	4.62±0.15 (73)	68.2±0.98 (75)	15.48±0.48 (146)	215.4±4.50 (118)	3.05±1.25 (258)
Three weeks	Control	6.40±0.02 (100)	92.5±1.28 (100)	10.85±1.26 (100)	178.0±1.70 (100)	1.10±0.05 (100)
	3.0 mg/L	4.85±0.15 (76)	76.4±1.26 (83)	15.20±0.46 (140)	210.0±2.20 (118)	2.98±0.15 (271)
	5.0 mg/L	4.20±0.28 (66)	62.5±0.58 (68)	16.78±0.48 (155)	225.5±1.25 (127)	3.12±0.08 (284)

Data are mean±S.E. of six replicates

Table 5: Levels of non-cellular blood constituents of freshwater fish *Hetropneustes fossilis* after 2 weeks exposure of sub-lethal dose and after two weeks withdrawal of treatment of alcoholic leaf extracts of *Lantana indica* plant

Exposure period	Experimental group	Serum total protein (g/dL)	Serum glucose (mg/dL)	Serum total lipid (g/L)	Serum total cholesterol (mg/dL)	Serum creatinine (mg/dL)
Two week	Control	6.32±0.04 (100)	94.0±1.36 (100)	10.85±0.06 (100)	180.0±1.65 (100)	1.12±0.18 (100)
	5.0 mg/L	5.15±0.15 (81)	72.5±1.25 (77)	14.72±0.38 (136)	190.5±3.50 (106)	2.45±0.18 (219)
Two weeks	Control	6.28±0.02 (100)	93.0±1.26 (100)	10.65±0.04 (100)	178.0±1.85 (100)	1.15±0.08 (100)
after withdrawal	Withdrawal	6.00±0.15 (96)	85.0±2.20 (91)	12.86±0.42 (121)	182.0±6.50 (102)	1.45±0.15 (126)

Data are mean±S.E. of six replicates

in the two experimental groups (Table 4). In the present study, a highly significant ($p<0.001$) hypoglycemic effect was more pronounced in leaf extract of *Lantana indica*, when compared with the control values (Table 4). Data resulted in Table 4 clearly refer to a slight dislipidamic effect as compared to the control levels (180.0±1.85), serum total cholesterol levels were significantly ($p<0.05$) increased. A highly significant ($p<0.001$) rise was observed in creatinine levels among fish *Heteropneustes fossilis* treated with the alcoholic leaf extract of *Lantana indica* after 1-3 weeks of administration (Table 4 and 5).

DISCUSSION

In the present studies, the exposed of different concentration of alcoholic leaf extract of *Lantana indica*, caused a detectable changes in fish behavior. In other species behavioral responses such as a progressive decrease in appetite, loss of condition and weakness in crossbred, mice, sheep, rat, goats, Guinea pigs and chicken (Vijjanand Tripathi, 1980; Vijjan and Parihar, 1983; Singh *et al*., 1985; Ibrahim *et al*., 1992; Ali, 1987) were observed after the exposure to the neem products, on the other hand the minor mortality may be due to the adaptation response of fish to this stressor.

In fishes, a change of the blood cell distribution has been correlated with the changes in environmental conditions (De Wilde and Houston, 1967; Gardner and Yevich, 1969). Our results indicated that administration of the *Lantana indica* leaf extract resulted in a significant decrease of the red blood cell counts, hemoglobin content and haematocrit value. The Supports to our results came from several previous studies concerning higher plants belonging to the family Meliaceae. Roa (1977) found a decrease in hemoglobin content in calves fed neem-seed cake. Ali (1987) observed a decrease in erythrocyte count, packed cell volume and haemoglobin content in goats and guinea pigs dosing with *Azadiracta indica* leaves.

This effect on freshwater fish *Heteropneustes fossilis* might have been achieved through failure or suppression of normal mechanisms promoting erythropoietin and/or deficiency of some factors required for the maturation of the red cell. The causes of leucopenia observed in the present study are supposed to be according to the degeneration, depression, depletion and destruction of the blood forming materials, by the *Lantana indica* leaf extract. Bhatt and Farswan (1992) stated that the application of sub-lethal concentrations of piscicidal plants, induced a decrease in the total number of erythrocytes, leucocytes, haemoglobin content and haematocrit value in the freshwater fish *Bariluid benclesis*.

A decrease in serum total proteins came together with the result of Selye (1950), who found that a great variety of systemic stressors including toxicants initially tend to diminish the total serum proteins, Ibrahim *et al*. (1992) observed a decline in serum total protein of Brown *Hisex*chicks fed *Azadiracta indica* ripe fruit. Hypoglycemic activity of exposed of leaf of *Lantana indica* may be due to the presence of a hypoglycemic agent acts on insulin secretion by B-cells of pancreas, since neem leaf extracts did not produce hypoglycaemia in totally pancreatomized rats, or animals made severely diabetic (Luscombe and Taha, 1974).

A close relationship between glucose and fatty acid metabolism, this help to explain the results of several investigators who have been shown that under certain conditions fat mobilization can be associated with diminution in glucose levels, (Masoro, 1977; Block and Vance, 1977), so we can speculate that the elevation of total lipid levels as a result of a diminution in glucose levels.

CONCLUSION

In conclusion, the leaf extracts of *Lantana indica* exposed against freshwater cat fish *Heteropneustes fossilis* is hazardous, Because plant products are less expensive easily available and easily soluble in water, may be used preferred over commercial pesticides.

REFERENCES

Ali, B.B., 1987. The toxicity of *Azadiraachtaindica* leaves in goats and guinea pigs. Vet. Hum. Toxicol., 29(1): 16-19.

APHA, 1992.Standard Methods for the Examination of Water and Waste Water. 18th Edn., American Public Health Association, American Water Works Association, Water Pollution Control Federation, Washington, DC, pp: 1268.

Arasta, T., V.B. Bais and P. Thakur, 1996.Effect of Nuvan on some biochemical parameters of Indian catfish *Mystusvittatus*.J. Environ. Res. Sec. A, 80: 80-83.

Arkin, M. and R.R. Cotton, 1963.Statistical Methods. 4th Edn.,Bermes and Noble Inc., NY, pp: 114.

Begum, S., S.M. Raza, B.S. Siddiqui and S. Siddiqui, 1995.Triterpenoids from the aerial parts of *Lantana camara*. J. Nat. Prod., 58: 1570.

Bhatt, J.P. and Y.S. Farswan, 1992.Haemolytic activity of piscicidal compounds of some plants to freshwater fish *Bariliusbendelisis*. J. Environ. Biol., 13(4): 333-342.

Block, K. and D. Vance, 1977. Control mechanisms in the synthesis of saturated fatty acids. Annu. Rev. Biochem., 46: 263-270.

Clerch, A. and J. Miale, 1970. Determination of blood cholesterol by the unitest system. Lat. Am. Pathol. Rev., 9: 115-120.

Dacie, J.V. and S.M. Lewis, 1968. Practical Haematology. 4th Edn., J. and A. Churchill, London.

De Wilde, M.A. and A.H. Houston, 1967. Haematological aspects of the three moacclimatory process in the rainbow trout, *Salmogaiirderi*. J. Fish Res. Bd. Can., 24: 2267-2281.

Frings, C.T., R.D. Fendley and C. Queen, 1972. Improved determination of total serum lipids by the sulfohhosph-vanillin reaction.Clin. Chem., 18: 673-680.

Gardner, G.R. and P.P. Yevich, 1969.Studies in the blood morphology of three estuarine Cypinodontiform fishes. J. Fish Res. Bd. Can., 26: 433-447.

Ibrahim, I., S.A. Omer, F.H.I. Ibrahim, S. Khalid and S. Adam, 1992.Experimental *Azadirachtaindica* in chicks. Vet. Hum. Toxicol., 34(3): 221-224.

Kirtikar, K.R. and B.D. Basu, 1961.Indian Medicinal Plants. S.N. Basu, Panini Office Bhuwaneshwariasrama, Bahadurganj, Allahabad, India, 1984.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Luscombe, D.K. and S.A. Taha, 1974. Pharmacological studies on the leaves of *Azadirachtaindica*. J. Pharm. Pharmacol., 26: 110-111.

Marston, A. and K. Hostettmann, 1985. Plant molluscicides. Phytochem., 24: 639-652.

Masoro, E.J., 1977. Lipids and lipid metabolism. Ann. Rev. Physiol., 39: 301.

Mian, L.S. and M.S. Mulla, 1992.Effects of parathyroid insecticides on non-target invertebrates in aquatic ecosystem. J. Agric. Entomol., 9(2): 73-98.

Oser, B.L., 1965. Hawks Physiological Chemistry. 14th Edn.,McGraw-Hill, New York.

Roa, B.S., 1977. Alkali treated neem (*Azadirachtaindica*) seed cake as a cattle feed. M.Sc. Thesis, Rohilkhand University, Bareilly.

Sastri, B.N., 1962. The Wealth of India. Council of Scientific and Industrial Research, New Delhi, 1: 425.

Selye, H., 1950. Stress and general adaptation syndrome. Br. Med. D., 1: 1383-1392.

Shaw, A.F., 1930. A direct method for counting the lecocytes, thromocytes and erythrocytes of birds. J. Ath. Bract., 33: 833.

Siest, G., J. Henny and F. Schielef, 1981. Interpretation of the Examine the Laboratory. In: Karger, S. (Ed.), Basel, pp: 206-223.

Singh, A. and R.A. Agarwal, 1988. Possibility of using latex of euphorbiales for snail control. Sci. Total Environ., 77: 231-236.

Singh, Y.P., H.S. Bahga and V.K. Vijjan, 1985. Toxicity of water extract of neem (*Azadirachtaindica*A. Juss) berries in poultry birds. NeemNewsl., 2: 17-18.

Singh, A., D.K. Singh, T.N. Mishra and R.A. Agarwal, 1996. Molluscicides of plant origin. Biol. Agric. Hortic., 13: 205-252.

Srivastava, M., V.K. Srivastava and A. Singh, 2007. Possibility of using different extracts of *Lantana indica* for control of mosquitoes and snail vectors. Nat. Prod. Rad., 6: 122-126.

Tsuyoshi, S.O. and K.B. Jaws, 1978.A simplified method of quantitating protein using the Biuret and phenol reagent.Anal.Bioch., 86: 193-200.

Vijjan, V.K. and H.C.N.S. Tripathi, 1980. A moteon the toxicity of neem (*A. indica*) seed cake in sheep. Environ. Biol., 3(2): 47-52.

Vijjan, V.K. and N.S. Parihar, 1983. Toxic effects of neem (*Azadirachtaindica*) seed cake feeding in rats. J. Environ. Biol., 41(1): 39-41.